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The principal objective of this grant is to target the nuclease activity of 1,10-phenanthroline-copper ion using synthetic peptides and genetically engineered DNA-binding proteins. These engineered nucleases could be used as artificial restriction enzymes and in the study of amino acid-nucleotide interactions essential for site specific binding.

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Synthetic Sequence Specific Nucleases

ONR N00014-86-K-0524

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and

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July 29, 1988

Research Objective: The goal of our studies is to target the nuclease activity of 1,10-phenanthroline-copper ion using synthetic peptides and genetically engineered DNA binding proteins.

Result (Year 2).

1. The *E. coli trp* repressor has been modified with a 1,10-phenanthroline derivative and converted into a site specific nuclease. See enclosed reprint, C.-h. B. Chen and D.S. Sigman "Chemical Conversion of a DNA-Binding Protein into a Site-Specific Nuclease," Science 237, 1197-1201 (1987).

2. Attempts to convert the cyclic-AMP binding protein into a site specific nuclease by a similar chemical modification procedure lead to the precipitation of the protein.

3. Two peptides have been synthesized for the purpose of targeting the nuclease activity. One peptide has the sequence:

```
                                                    77
Cys-Gln-Arg-Glu-Leu-Lys-Asn-Glu-Leu-Gly-Ala-Gly-Ile-Ala-Thr-Ile-
        85
Thr-Arg-Gly-Ser-Asn -
```

This sequence corresponds to the helix-turn-helix domain of the *trp* repressor. The N-terminal cysteine residue is not part of the sequence of the protein but has been appended to facilitate derivatization. The underlined residues at 77 and 85 have been substituted with valine and tryptophan, respectively. Structural studies have suggested that these changes increase DNA binding affinity.

Each peptide was a) dimerized by forming disulfide bonds at the N-terminal cysteine residue; and b) derivatized by 5-iodoacetyl-1,10-phenanthroline. The interaction of these various synthetic products with the *E. coli aro H* operator has been studied. This operator is one of three regulated by the *E. coli trp* repressor. The binding of the monomeric and dimeric unsubstituted peptides was studied using DNase footprinting. Under conditions in which the native protein binds with high affinity, none of these peptides showed any sequence specific interaction with the target DNA.

The scission of the *aro H* operator by the peptides derivatized with 1,10-phenanthroline was compared to the scission by the copper complexes of 1,10-phenanthroline and a 1,10-phenanthroline derivative with an amine linker arm. No difference in the digestion patterns could be observed between the different copper complexes.

4. The modified trp repressor described in our *Science* article is a potentially useful tool in the analysis of high molecular weight chromosomal DNA. In order to prove useful in chromosomal mapping studies, this semisynthetic nuclease must function within an agarose gel matrix. We are currently investigating the scission of the *E. coli* genome by the nuclease within the gel matrix to identify appropriate conditions for the scission reaction. It should be possible to isolate three distinct segments of DNA by pulsed field gel techniques.